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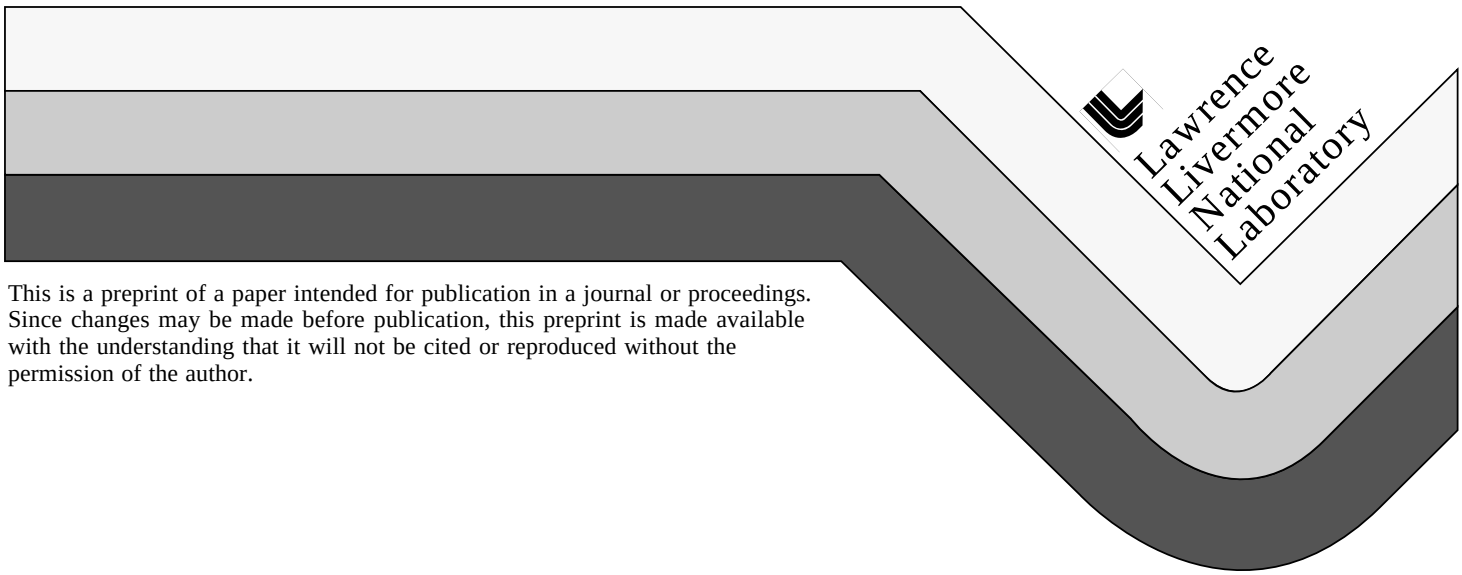

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TAXONOMIC AND DEVELOPMENTAL ASPECTS OF RADIOSENSITIVITY

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Abstract

Considerable information is available on the effects of radioactivity on adult and early life stages of organisms. The preponderance of data is on mortality after a single irradiation with relatively high doses. Unfortunately, because experiments were carried out under different conditions and for different time periods, the validity of comparing the results from different taxonomic groups is questionable. In general, the conclusions are that there is a relationship (1) between radioresistance to high doses of acute radiation and taxonomy of the organism, primitive forms being more radioresistant than complex vertebrates and (2) between radiosensitivity and developmental stage, early life stages being more sensitive than later stages. The first conclusion may be related to the capability of the organism to repopulate cells and to dedifferentiate and redifferentiate them; the second to the rate of cellular division and to the degree of differentiation. In question, however, is the relevance of the responses from high levels of acute radiation to that of the responses to long-term exposure to low levels of radiation, which are ecologically of more interest. Data from studies of the effects of acute and chronic exposure on development of gametes and zygotes indicate that, for some fishes and invertebrates, responses at the cellular and molecular levels show effect levels comparable to those observed in some mammals. Acute doses between 0.05 and 0.5 Gy and dose rates between 0.02 to 0.2 mGy/h appear to define critical ranges in which detrimental effects on fertility are first observed in a variety of radiosensitive organisms. To better understand inherent radiosensitivity, we need more information on the ability of cells to repopulate and differentiate and to prevent or repair damage to biological critical molecules, such as DNA, because these factors may alter significantly organisms' responses to radiation.

Introduction

The release of radioactive materials into the environment from planned or accidental discharges results in increased radiation exposure to humans and to native and domestic fauna and flora in our ecosystems. Considerable effort has been put forth to evaluate dose and dose-rate limits that protect humans [1-4]. Some efforts have been made to define limits to protect ecosystems [5, 6]. However, variation in environmental factors, such as temperature, salinity, light regime, and exposure media, results in further complexity of dose and safety assessments for populations. It is important to know the sensitivity to radiation of all living

organisms and not just humans, because the stability of ecosystems is vital for maintenance of the quality of human life. With information available on responses to irradiation and factors affecting radiosensitivity, organisms in ecosystems potentially at risk from accidental or planned releases of radioactivity can be identified. We will review the extensive database on mortality to ascertain the relationship between radiosensitivity and taxonomy and the less extensive database on responses of reproductive tissues and early life stages to define the relationship between radiosensitivity and development.

Abbreviations: Gy, gray; ppt, parts per thousand

Taxonomic Aspects of Radiosensitivity

Information is available on the effects of radiation on adult and early stages of organisms from different phyla and from different types of ecosystems. Although extensive data are available, there are entire phyla and groups within phyla for which there is no information; previous studies focused on a few species of mammals or of fishes. Most of the experiments were conducted to determine the responses to acute, high doses rather than chronic, low doses. However, it is the latter type of exposure that is more relevant to conditions that are present currently in the environment.

Acute Radiation Responses. A common experimental procedure in the past was to expose test organisms to a single irradiation using a relatively high dose to determine the dose at which mortality occurred. The response to the irradiation was expressed frequently as the median lethal dose or $LD_{50/30}$, which is the dose killing 50% of the population within 30 days. The period of 30 days was chosen probably because the small mammals used in early experiments had a good chance to survive if they did not die within this period. The ranges of responses of different groups of organisms to acute radiation are summarized in Table 1. There are large differences among groups in radiosensitivity, the ranges in response overlap, and lower taxonomic groups have lower radiosensitivity.

Representative data on mortality in adult mammals show that mammals have high radiosensitivity (Table 2). The $LD_{50/30}$ values are generally low, less than 10 Gy. Because the effects and mechanisms of action of acute radiation in some mammals and other model organisms have been investigated and the database updated periodically [1-4], information is available to provide a better understanding of responses to radiation.

The effects of acute radiation were examined using many fish species (Table 2). The range of lethal levels is from about 3.75 to 100 Gy. For some fishes, the effects for a specific total dose of decreasing the dose rate and of fractionating the dose were studied [17-20]. The results are similar to those for mammals. As the dose rates are decreased and the intervals between fractionated doses are increased, a greater total

Table 1. Summary showing ranges of LD_{50}s obtained from acute irradiation of organisms from different taxonomic groups.[1]

Group	Dose, Gy
Protista	30 - 30,000
Invertebrates	2.1 - 1,100
Vertebrates	
Fishes	10 - >600
Amphibians	7 - >22
Reptiles	3 - 40
Birds	5 - 20
Mammals	2.5 - 150
Plants	1.5 - >130

[1] The radiation units in references were converted to grays for comparative purposes and for some values are approximations.

dose is required to produce the same biological effects observed at high dose rates with no fractionation.

Radiosensitivity for nonvertebrate organisms was generally lower than that for vertebrate organisms and ranged widely within a given taxonomic group and among different taxonomic groups (Table 3). Microorganisms may require large doses to kill them. Some bacteria populations continue to form colonies at doses greater than 100 Gy, but deep-sea bacteria are reported to be more sensitive to irradiation than those from more shallow areas of the sea [29]. More recently an extremely radioresistant bacterium *Deinococcus radiodurans* was identified. This species is capable of surviving 5,000 to 30,000 Gy of ionizing radiation [30]. Also, some protozoans are relatively radioresistant. One of the most resistant appears to be the ciliate *Paramecium aurelia*, which is reported to have an LD_{50} greater than 1,000 Gy. Among multicellular organisms, sponges and hydroids are also relatively radioresistant. For many higher invertebrates, the LD_{50} values range from tens to hundreds of Gy. However, a value of

Table 2. LD_{50}s from acute irradiation of mammals and fishes.[1]

	Dose, Gy	References
Mammals		
Humans	3	[7]
Monkey	6	[7]
Dog	2.5	[7]
Swine	2.5	[7]
Hamster	6	[7]
Mouse	6.4	[7]
Rabbit	7.5	[7]
Bat	150	[7]
Pisces		
Goldfish	3.75 - 100	[8-10]
Mummichog	10 - 20	[11]
Tench	12 - 55	[12]
Guppy	23.5	[13]
Chinook salmon	25	[14]
Mosquitofish	37	[15]
Pinfish	50	[16]

[1] The radiation units in the references were converted to grays for comparative purposes and for some values are approximations. Also, the exposure times in some experiments differed from 30 days.

about 2.1 Gy was reported for adult grass shrimp [27], a dose that is lower than the LD_{50} for many mammals.

A relationship between radioresistance to high doses of acute radiation and taxonomy of the organism exists, but rules on increasing radiosensitivity with taxonomic position are not absolute. It must be noted that poikilotherms (animals such as fishes and invertebrates) do not maintain a constant internal body temperature, and cell-cycle times are generally more variable and much longer than those in mostmammals. Furthermore, similar radiosensitivities of related species cannot be assumed. Hoppenheit [31] showed that the LD_{50} of the amphipod *Gammarus zaddachi* is about 17 Gy, which is about half of that of *G. duebeni.* The males of the latter are less sensitive than the females, 39 and 35 Gy, respectively. In

addition, the

Table 3. LD_{50}s obtained from acute irradiation of invertebrate organisms from different taxonomic groups.

Group	Dose, Gy	References
Protozoa	> 1000	[21]
Coelenterata	20 - 120	[22]
Porifera		
Adult	20 - 120	[22]
Platyhelminthes	55	[22]
Annelida		
Adult	100 - >500	[23]
Mollusca		
Early life	11	[24]
Adult	50 - 500	[25, 26]
Crustacea		
Adult	2.1 - 1000	[27, 28]
Echinodermata	100	[25]

[1] The radiation units provided in references were converted to grays for comparative purposes and for some values are approximations. Also, the exposure times in some experiments differed from 30 days.

LD_{50} of the phylogenetically advanced crustacean *Callinectes sapidus* was reported to be about 566 Gy [28].

Chronic Radiation Responses. The chronic effects of radiation on mortality were assessed in some mammalian and nonmammalian species [1-6, 32, 33]. For invertebrates, some data are available on both freshwater and marine organisms. For adults of the blue crab *Callinectes sapidus,* dose rates greater than about 0.3 Gy/h for 70 d were required to cause death [34]. For juveniles of the clam *Mercenaria mercenaria* dose rates as high as about 0.37 Gy/h for 14 months only resulted in decreases in reproduction and growth [35]. For the freshwater cladoceran *Daphnia pulex,* Marshall [36] observed increased mortality rates for the population at dose rates >0.48 Gy/h, and for adults of the pond snail *Physa heterostropha,* decreased survival was reported at dose rates of 0.1 Gy/h [37]. For

fishes, Erickson [13] reported no increase in mortality of the guppy *Poecilia reticulata* exposed to 0.05 to 1 mCi mL^{-1} of tritium (total dose of 3.4 to 47 Gy).

Factors Modifying Radiation Responses. The mortality response may be modified by experimental conditions and by life stage and physiological state of the organism. The LD_{50} results may be confounded because experiments were carried out for time periods other than 30 d and under different exposure conditions. For example, for fishes (Table 2) the time of exposure was as low as 7 days [16] and as high as 60 days [14]. Also, experimental conditions are especially important when the mortality of poikilothermic species is examined. Temperature usually increases radiation sensitivity. The results from five studies of fishes indicate that low temperature slows the rate of development of lethal lesions and thus postpones death [10-12, 15, 17]. For marine organisms, salinity alters the response to irradiation. The LD_{50} of the mummichog *Fundulus heteroclitus* at 25 ppt, 15 ppt, and 5 ppt salinity was about 10, 15, and 20 Gy, respectively [11]. In field studies, the results may be confounded also by exposure to other contaminants, such as heavy metals, pesticides, and polyhalogenated organic compounds, which may reduce physiological fitness and increase radiosensitivity [6].

Other important variables not always controlled in early experiments were life stages, age, and sex of the organisms. It is well documented that sensitivity of rapidly dividing cells is greater than those of nondividing cells and that sensitivity of a dividing cell differs with the stage of division [32, 33]. If comparisons are made among experimental organisms of different life stages, age, or sex, it is highly likely that the overall rate of cell division would not be the same and would be reflected in the LD_{50}s obtained.

Another factor to consider is that two different species may have the same LD_{50}, but the shapes of the response curves may be quite different (Figure 1). A spread in the bell-shaped response curve results from a broad range in radiosensitivity within the species. Such a response becomes important under conditions of chronic exposure when selection of radioresistant individuals may result in subsequent generations having a higher LD_{50} because of decreased radiosensitivity.

Developmental Aspects of Radiosensitivity

The database on sensitivity to radiation during development includes results from irradiation of reproductive tissues as well as early life stages. When reproductive tissues are irradiated, damages incurred by the developing gametes may be

quantified by observing changes in the number and condition of primordial germ cells and

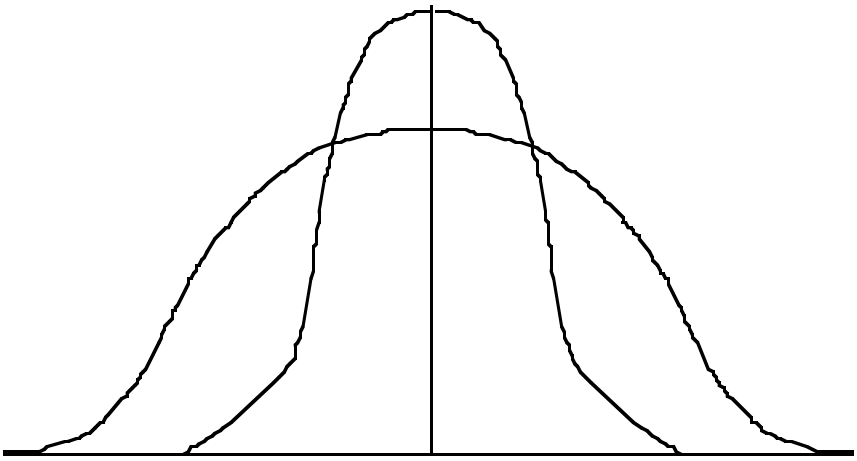

Figure 1. For the same LD_{50} value, the shape of species response curves from radiation may differ significantly.

developing gametes or in the size of the gonad. They may be quantified also by observing changes in the number of fertilized eggs produced and in the morphology and physiology of the developing embryos. When early life stages are irradiated, the effects quantified include the induction of abnormalities in the embryos and increases in mortality. Although the database is far from complete, sufficient information is available to permit some comparisons to be made.

An example of changes in sensitivity among developmental stages is available from the work of Ravera [26] who showed LD_{50} values for the four-cell, trochophore, veliger, and hippostage embryos of the gastropod *Physa acuta* to be about 11, 14, 49, and 110 Gy, respectively. Another example is that obtained by Welander *et al.* [14] and Welander [38] who determined that the LD_{50} for rainbow trout ranged from only about 0.5 Gy for gametes to about 150 Gy for the adult (Table 4).

A number of different biological responses can be used to assess radiosensitivity. Not only do these include mortality but also changes in morphology and physiology, in chromosome structure and function, in life span, etc. Information on sensitivity of different endpoints for the polychaete worm *Neanthes arenaceodentata* indicates that changes in chromosomal integrity (induction of sister chromatid exchange) and in reproductive success (fertility) occur at about the same dose (Table 5).

Acute Radiation Responses. Acute irradiation of reproductive tissues and early life stages results in changes in fertility, sterility, and normal development. The range

Table 4. Changes in the radiosensitivity of rainbow trout *Salmo gairdnerii* exposed to acute irradiation during development [14, 38].[1]

Stage in life cycle	LD_{50} (Gy)
Gamete	0.5 - 1.0
1 cell	0.58
32 cell	3.1
Germ ring	4.5 - 4.6
Eyed	4.1 - 9.0
Adult	15

[1] The radiation units in references were converted to grays for comparative purposes.[1]

Table 5. Sensitivity of different endpoints in the polychaete worm *Neanthes arenaceodentata*.

Dose, Gy	Endpoint	References
>0.3	DNA-strand breakage	[39]
>0.5	Reduced fertility	[23]
	Increased sister chromatid exchanges	[40]
>2	Increased chromosomal aberrations	[41]
>50	Sterility	[23]
>100	Lifespan reduction	[41]
>500	Mortality	[41]

[1] The radiation units provided in references were converted to grays for comparative purposes.

of acute irradiation that affects fertility in radiosensitive species appears to be between 0.05 and 0.5 Gy (Table 6). Among taxonomic groups, responses to radiation on reproductive tissues are better documented for fishes and small mammals than for invertebrates. Effects on gametes of fishes were performed, and the results were reviewed in Egami and Ijiri [49]. For example, effects of acute radiation on female

germ cells were observed after exposures to doses of acute radiation as low as 2.5 Gy. Welander *et al.* [14] found that counts of primordial germ cells in the Chinook salmon *Oncorhynchus tschawytscha* exposed to 2.5 Gy from a x-ray source were 10% of control values. Male germ cells of the medaka *Oryzias latipes* were studied extensively by Egami and co-workers, who found a temporary reduction in testicular weights after exposure to a range from about 1 to 20 Gy.

In fishes, irradiation not only may retard development but also alter morphological and physiological characteristics. Some of the criteria used to evaluate effects include hatching success, embryo mortality, and frequency of morphological abnormalities in embryos, larvae, young, and adults [32, 33]. Other responses noted were that the somatic damage in Chinook salmon was proportional to the amount of radiation received and that the greatest amount of damage was in tissues growing and dividing rapidly. From the differences in sensitivities shown by successive early stages of development, it appears that different processes are involved and that these processes are in progress at various times prior to morphological evidence of the organogenesis.

Chronic Radiation Responses. Dose rates that resulted in significant changes to fertility in invertebrates had a larger range of values than in fishes and mammals; the range for the invertebrates was from 0.07 to 550, for fishes <0.6 to 4.2, and for mammals 0.023 to 0.07 mGy/h (Table 7). Because the database for fishes and invertebrates is small, any conclusions about the significance of the differences may not be valid. In a summary of data for mammals [3], the reader is cautioned that responses are dependent on the developmental stage of the gonadal tissue at the time and duration of the irradiation, and for any species the range in sensitivity may be large. Although such changes in sensitivity are not as well documented in other taxonomic groups, it is likely to be an important factor.

The dose rates known to cause sterility in different species have a large range—0.23 to 1400 mGy/h. Differences occur because the processes of gametogenesis are not the same from species to species, and for a given species, the response of male and female reproductive tissues may differ. In general, the testis is more radioresistant than the ovary. In some species, sterility requires dose rates and doses to the testis larger than those causing adult mortality, and these may cause only temporary sterility. Recovery of gonads from radiation damage may reflect differences in radioresistance of the stem cell population between sexes and among species. Another factor that differs greatly from species to species is the differences in doses and dose rates causing decreased fertility and sterility. In the

p o l y c h a e t e w o r m *Neanthes*

Table 6. Comparison of sensitivity of reproductive tissues of invertebrates, fishes, and mammals exposed to acute irradiation (Gy). The doses for fertility are those at which significant changes were noted and for sterility were for when the response was noted.[1]

	Fertility	Sterility	References
Invertebrates			
Neanthes arenaceodentata (polychaete worm, adults)	0.5	50	[23]
Gammarus duebeni (amphipod, adults)	2.2	--	[31]
Artemia salina (brine shrimp, juveniles)	9	21	[42]
Diaptomus clavipes (copepod, embryos)	10	--	[43]
Crepidula fornicata (slipper limpet, larvae)	20	--	[44]
Physa acuta (freshwater snail, adults)	20	1000	[26, 45]
Fishes			
Oryzias latipes (medaka, adult males)	5	--	[46]
Oncorhynchus tschawytscha (Chinook salmon, embryos)	2.5	--	[14]
Salmo gairdnerii (rainbow trout, 29-d embryos)	6	--	[47]
Mammals			
Mice (LD_{50}, primordial follicles)	0.1	1	[48]
Rat (LD_{50}, primordial follicles)	0.7	8	[48]
Monkey	10	20	[48]
Human male	0.15	3.5 - 6	[48]
Human female	0.06	2.5 - 6	[48]

[1] The radiation units provided in references were converted to grays for comparative purposes and for some values are approximations.

Table 7. Comparison of sensitivity of reproductive tissues of invertebrates, fishes, and mammals exposed chronically to radiation (mGy / h). The doses for fertility are those at which significant changes were noted and for sterility were for when the response was noted.[1]

Invertebrates	**Fertility**	**Sterility**	**References**
Pollicipes polymerus (goosebarnacle, larvae)	0.07	--	[50]
Neanthes arenaceodentata (worm, single generation)	0.19	20	[51]
Ophyrotrocha diadema (worm, seven generations)	3.2--		[52]
Daphnia pulex (water flea, multiple generations)	550	1400	[36]
Fishes			
Ameca splendens (--, single generation)	<0.6	0.6	[53]
Poecilia reticulata (guppy, single generation)	1.713		[54]
Oryzias lalipes (medaka, adult males)	2.8840		[46]
Oncorhynchus tschawytscha (Chinook salmon, embryos)	4.2--		[55]
Gambusia affinis	13		[56]
Mammals			
Human male	0.050	0.23	[48, 57]
Human female	0.023	--	[48, 57]
Male Dog	0.070	0.17	[48, 57]

[1] The radiation units provided in references were converted to grays for comparative purposes and for some values are approximations.

arenaceodentata, the difference is two orders of magnitude whereas for male dogs it is a factor of about two.

Reproductive success for a given species may be related not only to its sensitivity to radiation during gametogenesis and early development but also its reproductive strategy [32, 33]. For example, in a highly fecund species, the survival of early life stages may be very low, and the loss of abnormal embryos induced from radiation exposure may be masked completely by those lost from other ecological factors, such as food limitation and predation. Other important factors of reproductive strategy, in addition to the total number of gametes produced, their rate of division, and their sensitivity, are gametogenesis parameters, such as the time between production and release of gametes, the time to sexual maturity, and the brooding of young. The time between the formation of primary germ cells and the release of mature gametes becomes important in long-lived species exposed to chronic irradiation. In the case of marine mammals and some fishes, if repair of radiation damage does not occur, the dose to reproductive tissues may be integrated over a period of tens of years. Unfortunately, in many nonmammian organisms the processes involved in radiosensitivity and in gametogenesis and reproductive strategies are not known.

The data most relevant to protection of ecosystems through limit setting are those values obtained from developmental responses rather than mortality because high radiosensitivity of gonadal tissues and early life stages affects reproductive success directly. Also, if for the same group of species the radiosensitivity of adults as measured by mortality is compared to that of early stages as measured by developmental changes, it is evident that the radiosensitivity during development may not be in the same taxonomic relationship as that of the mortality of adults. Also, the responses of special interest are the low ones resulting in decreased fertility. For invertebrates the low values are in about the same range as those for some fishes and mammals, indicating that at the cellular and molecular levels, radiosensitivity may not differ much if similar stages in gametogenesis are exposed.

Reproductive success of a species in natural ecosystems is affected also by the changes in the population gene pool from multigeneration exposures to radiation. At most radioactivity-contaminated ecosystems, the exposure to the biota is chronic and at low levels, resulting in multigeneration exposures. However, data available on the effects of this type of irradiation on reproductive success are limited [6, 32, 33]. The database from laboratory studies contains

information from only a few studies that were on multigenerations. The duration of most studies was for less than a complete life cycle, and the stages in the life cycle irradiated were not always comparable. The database from field studies includes results from multigeneration investigations, but the results from many of these studies were confounded by the presence in the ecosystem of contaminants other than radioactivity [6]. Effects of multigeneration exposure becomes important because the dose-response curves for specific species may differ greatly (see Fig. 1). It can be expected that selection of radioresistant individuals will occur upon continuous exposure, and species having a broader range in sensitivity may have a greater potential for survival.

Inherent Radiosensitivity Factors

The information reviewed indicates that responses during reproduction and development, which may reflect changes at cellular and molecular levels, represent better the inherent radiosensitivity of the species than the mortality responses of adults. We will consider inherent radiosensitivity factors to be those that are controlled by the genetic make up of the organism and that determine basic developmental processes and pathways as well as biological repair processes. Although environmental conditions, such as temperature, salinity, contaminants, and exposure conditions, are known to alter the observed radiosensitivity, there are some parameters that most likely reflect inherent radiosensitivity of an organism. Such parameters include the (1) nuclear material content, (2) cell repopulation, (3) tissue and organ regeneration, and (4) biological repair. Concerning biological repair, it is important to consider how it may modify the responses of organisms to low levels of irradiation and how that response may affect our application of the linear-no-threshold theory for prediction of radiation damage.

Nuclear Material Content. The content of nuclear material in cells was shown in early experiments to be related to radiosensitivity in both plants and animals. Investigations of the relationship between average interphase nuclear volume (INV) and responses to acute radiation were performed on large number of plants in the 1960s by Sparrow and coworkers [7]. They showed that plants with large nuclear volumes were generally more sensitive than plants with small nuclear volumes and that woody species are about twice as sensitive as herbaceous species for a given INV. This relationship was used subsequently to predict the

sensitivity of plants to chronic irradiation. Additional work on INV and sensitivity was performed on amphibians, where a similar relationship was determined [7].

Cell Repopulation. The ability of cells to repopulate themselves to replace cells damaged by injury or by radiation and to orchestrate tissue and organ regeneration is undoubtedly an inherent trait of organisms. In tissue repair, a number of growth-factor genes are induced and help direct tissue repair, but the molecular signals that initiate the process are not established completly but are currently under investigation [58]. The cells involved in repopulation and division may be cells that never differentiated, such as primordial germ cells, stems cells, and other types of cells that were "set aside" during early development [59], or cells that had dedifferentiated or transdifferentiated [60]. If organisms have the capacity to replace cells, the radiation damage observed at the whole organism level may be masked.

Tissue and Organ Regeneration. Tissue and organ regeneration has been demonstrated in many more primitive organisms, some of which appear to be relatively radioresistant as adults. The ability of cells to repopulate is undoubtedly an important component of regeneration. Both the abilities to repopulate cells and regenerate structures are related to basic developmental processes and pathways and are important components of recovery from radiation damage.

The methods of cell specialization by embryos from different phylogenetic groups were studied and shown to be diverse [61]. But now studies of developmental processes and pathways appear to be entering a crucial period of discovery in model organisms, such as bacteria [62] yeast [63], sea urchins [64, 65], worms [66], and zebrafish [67]. Cell division is known to be followed by cell specialization or specification, a process by which the fate of cells is established and the consequences are the installation of differential patterns of gene expression. It is important to know the mechanisms of cell specialization, i.e., to know how one cell divides into two cells with the same genome but with distinct functions, eventually becoming part of specialized tissues and organs.

The study of the mechanism of cell specialization during sporulation in *Bacillus subtilus* provides an indication of the current trends in research. In this bacterium, the process involves an initial asymmetrical division followed by the activation of different genes [63]. Involved in asymmetrical division is a protein initiating a cascade of biochemical reactions on one side of the cell membrane, and this leads to the activation of a series of genes that convert the forespore into a spore. Such proteins have been given the name of "signaling" proteins. Not only

are they known to play a role in sporulation in *B. subtilus* but also in cell fate in the algae *Volvox carteri* and in the development of the nervous system of the fruit fly [63]. However, ultimate differences in the cells must depend on the activation of distinct genes in each cell. Differential activation is considered to be accomplished through a series of transcriptional factors called sigma factors. These sigma factors are proteins reported to bind to sites near the beginning of genes to initiate messenger RNA synthesis. The ability to produce signaling proteins and sigma factors during early life and adult stages may account in part for differences among organisms in their capabilities to repopulate damaged cells and to regenerate tissues and organs.

Biological Repair. Inherent radiosensitivity is also related to the biological repair capability of cells. Biological repair consists of repair of nuclear as well as cytoplasmic materials. The main focus of repair in the nucleus is on the processes involved in the repair of DNA; that of cellular repair is on the group of enzymes that are involved in the prevention of and in the repair of damaged constituents within the cytoplasm.

The ability of cells to repair radiation damage was noted early on when organisms were observed to often show reduced sensitivity when exposed to fractionated doses [1, 32, 33]. The conclusion was made that splitting the dose allows repair processes to reduce the damage. Currently, there is sufficient information to conclude that repair mechanisms are widely distributed and are important to radiosensitivity responses. The mechanism receiving the most attention is DNA repair, and an extensive database is available on the genes involved and the processes occurring in a wide variety of organisms [3, 4].

One of the most remarkable capability to repair DNA damage was reported for the bacterium *Deinococcus radiodurans*, which is capable of surviving up to 30,000 Gy of ionizing radiation [30]. Such a dose shatters the organism's chromosomes into hundred of fragments, yet because of an extraordinary ability to recover, due in part to its efficient DNA repair machinery, the organism survives. Because such high radiation doses are not found normally in the environment, it was proposed that the radiation resistance may be the result of its ability to repair its DNA after severe dehydration.

One aspect of DNA repair that is progressing rapidly is the identification of "checkpoints"—specific times within the cell cycle during which progression through the cycle can be delayed in response to either DNA damage or to incompletion of prior cell cycle events, such as DNA replication [68]. It is well

documented that treatment of dividing cells with radiation causes a pause in the G_2 phase of the cell cycle, and when the pause is absent, the cells are more sensitive to radiation. Recently, many proteins in the checkpoint pathways were identified in yeast [68, 69]. Mitotic checkpoints require three distinct functions: (1) a detection system to determine the change in DNA structure, (2) a signal pathway to transmit this information, and (3) an effector mechanism to interact with the cell-cycle machinery. It is expected that the progress made in the genetic analysis of yeast will yield identification of biochemical markers for checkpoints, which can be used to characterize responses in other organisms.

Indirect damage in genetic material from free radicals produced in cells from radiation is a likely occurrence [32, 33]. Defense mechanisms against the production of free radicals formation were reviewed by Giulio *et al.* [70] who were concerned primarily about xenobiotic molecules, such as quinone, aromatic nitro compounds, aromatic hydroxylamines, bipyridyls, and certain metal chelates. They proposed that "antioxidant defenses are of three general classes and include water soluble reductants (glutathione, ascorbate, urate), fat soluble vitamins (alpha tacopherol, beta carotene) and enzymes (glutathione peroxidase, catalase, superoxide dismutase)." The enzymes are of special interest because they are inducible under conditions of oxidative stress.

Much of the indirect damage caused by ionizing radiation is considered to be due to the hydroxyl radical, which is one of the most reactive radical known. In the presence of transition metal catalysts, superoxide and hydrogen peroxide will react to form the hydroxyl radical. The hydroxyl radical reacts with extremely high rate constants with virtually all types of molecules found in living cells. It is known to inactivate enzymes and, thus, is capable of wide ranging cellular damage. The action of the superoxide free radical, a free radical arising from the univalent reduction of molecular oxygen, is important also, because after it is formed it can be dismutated by superoxide dismutases, which are present in all living oxygen-tolerant organisms.

Because the kinds and quantities of antioxidant-defense enzymes induced may differ with species, radiosensitivity in the presence of oxygen may be affected. Therefore, to have a complete understanding of species tolerance to low levels of radiation, it is necessary to consider the capability of the species to reduce concentrations of free radicals by antioxidants. Although little information is known about the role of antioxidants in preventing radiation damage in fishes and

invertebrates, some information is available on methods to quantify oxidative stress-related responses induced in these organisms from xenobiotic chemicals [70].

Adaptive Responses to Low Levels of Radiation

More attention has been directed recently to responses to low levels of radiation and what has been called an "adaptive response." This response to low doses, which remains for several hours in mammals, is sometimes referred to as stress response or response to genotoxic stress, and it may affect our use of the linear-no-threshold theory for radiation damage. Considerable data have accumulated indicating that low doses of radiation may result in changes in the cells, reflecting an ability to adapt to the effects of radiation [6, 32, 33]. In the UNSCEAR report [4], it is noted that the "conventional estimates of the risks of stochastic effects of low doses of ionizing radiation many have been overstated because no allowance was made for the adaptive response."

Reported manifestations of adaptive responses in mammals are accelerated growth, increased reproductive ability, extended life span, stimulation of the immune system, and reduced incidence of radiation-induced chromosomal aberrations and mutations. Some of the mechanisms proposed to be involved in the adaptive response and might be expected to be reflected in radiosensitivity responses were the following [4]:

"(a) the effects of radiation on the up-regulation of genes and their influence on cell cycle kinetics;

(b) the identification of activated genes and their enzyme products specifically involved in radiation-induced DNA repair;

(c) the relationship between radiation-induced repair genes and those activated by other mutagens;

(d) the ability of cells to remove toxic radicals;

(e) the activation of membrane receptors and the release of growth factors;

(f) the effects of radiation on the proliferative response to mitogens."

These factors may come into play at low doses and low dose rates, which are characteristic of many of the conditions found in the environment.

Although investigations of the adaptive response have not been investigated in flora and fauna of concern in ecosystems, some information does exist on DNA repair and on enzymes involved in the reduction of free radicals. DNA-strand breakage was investigated in freshwater fishes [71, 72] and in a marine

bivalve and a polychaete worm [39]. Results from experiments using DNA-strand breakage as the endpoint indicate that after these organisms are irradiated, DNA-strand breakage is repaired. However, the time course of repair is much slower in nonmammals than mammals; the time of repair takes days rather than hours. Also, little is known about the fidelity of the repair. Another important consideration is that in mammals there is evidence that the lesions induced by radiation may also be induced by some other toxic agents. These not only include physical agents but also chemical materials. Before the consequence of low doses of radiation on nonmammalian species can be elucidated, allowance for uncertainty should be considered in predictions of effects on the environment. The adaptive response and its effect on interactions among contaminants in the environment becomes a research area that needs to be addressed.

Summary

The ranges of the LD_{50} responses of different taxonomic groups of organisms to acute radiation indicate that there are large differences among groups in radiosensitivity and that lower taxonomic groups have lower radiosensitivity. Lists of median lethal doses causing mortality are to be interpreted with caution when making comparisons about radiosensitivity of specific species within a group and, in some cases, of species from different phyla. Also, because the responses that are elicited at high doses may be entirely different from those at low doses, erroneous conclusions may be drawn when short-term mortality results are used to approximate long-term impacts. Because of uncertainties associated with factors, such as environmental variables and physiological condition, the lower limit of radiation inducing mortality in many species is most likely still undefined.

Responses to radiation during reproduction and development indicate that early life-history stages are more sensitive than adults and reflect changes occurring at the cellular and molecular levels. Comparison of effects on reproductive tissues from different groups of organisms indicates that radiosensitivity responses may be modified by environmental conditions and by types of irradiation. Comparison of the effects of radiation on fertility and sterility in different groups of sensitive mammals, fishes, and invertebrates appears to indicate that there is a lower threshold level of doses, ranging from about 0.05 to 0.5 Gy, and dose rates, ranging from about 0.02 to 0.2 mGy/h, below which no adverse effects are detected.

The inherent radiosensitivity, which is genetically controlled in an organism, is a critical factor in determining the responses to radiation. The responses observed may be modified by environmental factors and physiological condition but reflect fundamental processes and pathways in reproduction and development and the ability to prevent or repair damage to biologically critical molecules. Even though deleterious responses are not detected generally above the apparent lower threshold level, effects may be occurring that cannot be quantified with our current state of technology. Because rapid process is being made in the understanding of developmental processes and pathways and in biological repair mechanisms, information may be available soon that will allow us to understand factors determining inherent radiosensitivity and to identify endpoints or biomarkers to be used to set better standards for the protection of humans and the environment.

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References

[1] ICRP (1979). *Radionuclide Release into the Environment: Assessment of Doses to Man*. International Commission of Radiological Protection, ICRP Publication 29, Pergamon Press, New York, NY

[2] ICRP (1991). *Recommendations to the Commission*. International Commission of Radiological Protection, ICRP Publication 60, Pergamon Press, New York, NY

[3] UNSCEAR (1993). *UNSCEAR 1993 Report to the General Assembly with Scientific Annexes*. United Nations Scientific Committee on the Effects of Atomic Radiation, United Nations, New York, NY

[4] UNSCEAR (1994). *UNSCEAR 1994 Report to the General Assembly, with Scientific Annexes*. United Nations Scientific Committee on the Effects of Atomic Radiation, United Nations, New York, NY

[5] IAEA (1988). *Assessing the Impact of Deep Sea Disposal of Low Level Radioactive Waste on Living Marine Resources*. Technical Reports Series No. 288, International Atomic Energy Agency, Vienna, Austria

[6] NCRP (1991). *Effects of Ionizing Radiation on Aquatic Organisms*. National Council on Radiation Protection and Measurements, NCRP REPORT No. 109, Bethesda, MD

[7] Rice, TR and Baptist, JP (1974). Ecologic effects of radioactive emissions from nuclear power plants, in *Human and Ecological Effects of Nuclear Power Plants*. Sagin, LA, Ed., Thomas, CC, Springfield, IL, pp. 373-439

[8] Etoh, H, Hyodo-Taguchi, Y and Maruyama, T (1974). Mortality response of goldfish after exposure to fast neutrons. Journal of Radiation Research, 9: 141

[9] Shechmeister, IL, Watson, LJ, Cole, VW and Jackson, LL (1962). The effect of x-irradiation of goldfish: Effect of x-irradiation on survival and susceptibility of the goldfish, *Carassius auratus*, to infection by *Aeromonas salmonicida* and *Gyrodactylus spp.* Radiation Research, 16: 89-97

[10] Hyodo-Taguchi, Y (1965). Effect of x-irradiation on the intestinal epithelium of the goldfish *Carassius auratus:* II. Influence of temperature on the development of histopathological changes in the intestine. Radiation Research, 24: 133-141

[11] Angelovic, JW, White Jr, JC and Davis, EM (1969). Interactions of ionizing radiation and temperature on the estuarine fish, *Fundulus heteroclitus*, in *Proceedings of the 2nd National Symposium on Radioecology*. Nelson, DJ and Evans, FC, Eds., CONF-670503, US Atomic Energy Commission, Washington, DC, pp. 131-141

[12] Lockner, D, Petterson, A and Ericson, U (1972). Radiosensitivity of haemopoiesis in fish studies at 18^{o} and 10^{o} C. Journal of Radiation Biology, 21: 559-572

[13] Erickson, RC (1973). Effects of chronic irradiation by tritiated water on *Poecilia reticulata*, the guppy, in *Proceeding of the 3rd National Symposium on Radioecology*. Nelson, DJ, Ed., CONF-710501, US Atomic Energy Commission, Washington, DC, pp., 1091-1099

[14] Welander, AD, Donaldson, LR, Foster, RF, Bonham, K and Seymour, AH (1948). The effects of roentgen rays on the embryos and larvae of the Chinook salmon. Growth, 12: 203-242

[15] Blaylock, BG and Mitchell, TJ (1969). The effect of temperature on the dose response of *Gambusia affinus affinus* from two natural populations. Radiation Research, 40: 503-511

[16] Engel, DW, Angelovic, JW and Davis, EM (1967). *Effects of Acute Irradiation on the Blood of Pinfish.* US Fish and Wildlife Service, Circular 270

[17] Egami, N and Etoh, H (1966). Effects of temperature on the rate of recovery from radiation-induced damage in the fish, *Oryzias latipes,* at different temperatures. Radiation Research, 27: 630-637

[18] Etoh, H and Egami, N (1967). Damage accumulation and recovery in the fish, *Oryzias latipes*, exposed to fractionated or protracted radiation at different temperatures. Radiation Research, 32: 884-891

[19] Egami, N (1969). Kinetics of recovery from injury after whole-body x-irradiation of the fish, *Oryzias latipes*, at different temperatures. Radiation Research, 37: 192-201

[20] Chee, PC, Vetter, RJ and Atchison, GJ (1979). Effect of dose rate on survival time in the fathead minnow, *Pimephales promelas.* Radiation Research, 77: 98-102

[21] Bacq, ZM and Alexander, P (1961). *Fundamentals of Radiobiology*. Pergamon Press, Oxford

[22] Polikarpov, GG (1966). *Radioecology of Aquatic Organisms*. Reinhold, New York, NY

[23] Harrison, FL and Anderson, SL (1994). The effects of acute irradiation on reproductive success of the polychaete worm *Neanthes arenaceodentata*. Radiation Research, 137: 59-66

[24] Blaylock, BG and Trabalka, JR (1978). Evaluating the effects of ionizing radiation on aquatic organisms, in *Advances in Radiation Biology,* Vol. 7. Lett, JT and Adler, H, Eds., Academic Press, New York, NY, pp. 103-152

[25] Templeton, WL, Nakatani, RE and Held, EE (1971). Radiation effects, in *Radioactivity in the Marine Environment.* National Academy of Sciences, Washington, DC, pp. 223-239

[26] Ravera, O (1967). The effect of x-rays on the demographic characteristics of *Physa acuta* (Gastropoda: Basommatophora). Malacologia, 5: 95-109

[27] Engel, DW, Shelton, MG and White Jr, JC (1974). The effect of ionizing radiation and salinity on the grass shrimp *Palaemonetes pugio*. Radiation Research, 59: 72 (abstract)

[28] Chipman, WA (1972). Ionizing radiation, in *Marine Ecology*, Vol. 1. Kinne, O, Ed., John Wiley / Interscience, New York, NY, pp. 1579-1657

[29] Yayanos, AA and Gomez, LS (1983). Gamma ray inactivation of deep-sea bacteria from ocean depths of 1900 to 10,500 m. Radiation Research Society (abstract)

[30] Daley, MJ and Minton, KW (1995). Resistance to radiation. Science, 270: 1318

[31] Hoppenheit, M (1973). Effects of fecundity and fertility of single sub-lethal x-irradiation of *Gammarus duebeni females*, in *Radioactive Contamination of the Marine Environment.* International Atomic Energy Agency, Vienna, Austria, pp. 479-486

[32] Woodhead, DW (1984). Contamination due to radioactive materials, in *Marine Ecology*, Vol. 5. Kinne, O, Ed., John Wiley and Sons, New York, NY, pp. 1111-11287

[33] Anderson, SL and Harrison, FL (1986). *Effects of Radiation on Aquatic Organisms and Radiobiological Methodologies for Effects Assessment*. US. Environmental Protection Agency, EPA 520-1-85-016

[34] Engel, DW (1967). Effects of single and continuous exposures of gamma radiation on the survival and growth of the blue crab, *Callinectes sapidus*. Radiation Research, 32: 685-691

[35] Baptist, JP, Wolfe, DA and Colby, DR (1976). Effects of chronic gamma radiation on the growth and survival of juvenile clams (*Mercenaria mercenaria*) and scallops (*Argopecten irradians*). Health Physics, 30: 79-83

[36] Marshall, JS (1962). The effects of continuous gamma radiation on the intrinsic rate of natural increase of *Daphnia pulex*. Ecology, 43: 598-607

[37] Cooley, JL and Miller Jr, FL (1971). Effects of chronic irradiation on laboratory populations of the aquatic snail *Physa heterostropha*. Radiation Research, 47: 716-724

[38] Welander, AD (1954). Some effects of x-irradiation of different embryonic stages of the trout (*Salmo gairdnerii)*. Growth, 18: 227-255

[39] Martinelli, RE, Knezovich, JP and Harrison, FL (1990). Radiation-induced DNA strand breakage and repair in the marine polychaete *Neanthes arenaceodentata*. Marine Environmental Research, 28: 329-332

[40] Harrison, FL, Rice Jr, D, Moore II, D and Varela, M (1987). Effects of radiation on frequency of chromosomal aberrations and sister chromatid exhange in *Neanthes arenaceodentata*, in *Oceanic Processes on Marine Pollution*. Vol. 1. Capuzzo, JM and Kester, DR, Eds., Krieger, Malabor, FL, pp. 146-156

[41] Anderson, SL, Harrison, FL, Chan, G and Moore II, DH (1990). Comparison of cellular and whole-animal bioassays for estimation of radiation effects in the polychaeta worm *Neanthes arenaceodentata* (Polychaeta). Archives of Environmental Contamination and Toxicology, 19: 164-174

[42] Holton, RL, Forster, WO and Osterberg, CL (1973). The effect of gamma irradiation on the reproductive performance of *Artemia* as determined by individual pair matings, in *Proceedings of a Symposium on Radiation in Ecosystems*. Oak Ridge, TN, US Atomic Energy Commission, Washington, DC, pp. 1191-1197

[43] Gehrs, CW, Trabalka, JR and Bardill, EA (1975). Sensitivity of adult and embryonic calanoid copepods to acute ionizing radiation. Radiation Research, 63: 382-385

[44] Greenberger, JS, Pechenik, J, Lord, A, Gould, L, Naparstek, E, Kase, K and Fitzgerald, TJ (1986). X-irradiation effects on growth and metamorphosis of gastropod larvae (*Crepidula fornicata*): A model for environmental radiation teratogenesis. Archives of Environmental Contamination and Toxicology, 15: 227-234

[45] Ravera, O (1966). Effects of x-irradiation on various stages of the life cycle of (*Physa acuta,* Daparnaud) a fresh-water gastropod, in *Proceedings of a Symposium on Disposal of Radioactive Wastes into Seas, Oceans, and Surface Waters.* International Atomic Energy Agency, Vienna, Austria, pp. 799-808

[46] Hyodo-Taguchi, Y (1980). Effects of chronic gamma-irradiation on spermatogenesis in the fish, *Oryzias latipes*, with special reference to

regeneration of testicular stem cells, in *Radiation Effects on Aquatic Organisms.* Egami, N, Ed., University Park Press, Baltimore, MD, pp. 91-104

[47] Konno, K (1980). Effects of gamma-irradiation on the gonads of the rainbow trout, *Salmo gairdnerii irideus*, during embryonic stages, in *Radiation Effects on Aquatic Organisms.* Egami, N, Ed., University Park Press, Baltimore, MD, pp. 129-133

[48] UNSCEAR (1992). *UNSCEAR 1992 Report to the General Assembly with Scientific Annexes*. United Nations Scientific Committee on the Effects of Atomic Radiation, United Nations Publication, United Nations, New York, NY

[49] Egami, N and Ijuri, KI (1979). Effects of irradiation on germ cells and embryonic development in teleosts. International Review of Cytology, 59: 195-248

[50] Abbott, DT and Mix, M (1979). Radiation effects of tritiated seawater on development of the goosebarnacle, *Pollicipes polymerus*. Health Physics, 36: 238-287

[51] Harrison, FL and Anderson, SL (1994). Effects of chronic irradiation on the reproductive success of the polychaete worm, *Neanthes arenaceodentata*. Radiation Research, 140: 401-409

[52] Knowles, JF and Greenwood, LN (1994). The effects of chronic irradiation on the reproductive performance of *Ophryothrocha diadema* (Polychaeta, Dorvilleidae). Marine Environmental Research, 38: 207-224

[53] Woodhead, DS, Barker, CJ and Rackham, BD (1983). The effects of chronic gamma radiation on experimental fish populations: A preliminary account, Abstract in *Biological Effects of Low-level Radiation*. International Atomic Energy Agency, Vienna, Austria, pp. 646-648

[54] Woodhead, DS (1977). The effects of chronic irradiation on the breeding performance of the guppy, *Poecilia reticulata* (Osteichthyes: Teleostei). International Journal of Radiation Biology, 32: 1-22

[55] Donaldson, LR and Bonham, K (1964). Effects of low-level chronic irradiation of Chinook and coho salmon eggs and alevins. Transactions of the American Fisheries Society, 93: 333-341

[56] Trabalka, JR and Allen, CP (1977). Aspects of fitness of a mosquitofish *Gambusia affinis* population exposed to chronic low-level environmental radiation. Radiation Research, 70: 198-211

[57] UNSCEAR (1982). *UNSCEAR 1982 Report to the General Assembly, with Scientific Annexes*. United Nations Scientific Committee on the Effects of Atomic Radiation, United Nations Publication, United Nations, New York, NY

[58] Khachigian, LM, Lindner, V, Williams, AJ and Collins, T (1995). EGR-1-induced endothelial gene expression—a common theme in vascular injury. Science, 271: 1427-1431

[59] Davidson, EH, Peterson, KJ and Camerson, RA (1995). Origin of bilaterian body plans: Evolution of developmental regulatory mechanisms. Science, 270: 1319-1325

[60] Patapoutian, A, Wold, BJ and Wagner, RA (1995). Evidence for developmentally programmed transdifferentiation in mouse esophageal muscle. Science, 270: 1818-1820

[61] Davidson, EH (1990). How embryos work: A comparative view of diverse modes of cell fate specification. Development, 108: 365-389

[62] Arigoni, F, Pogliano, D, Webb, CD, Stragier, P and Losick, R (1995). Localization of protein implicated in the establishment of cell type to sites of asymmetric division. Science, 270: 637-643

[63] Roush, W (1996). Defining the first steps on the path toward cell specialization. Science, 270: 578-579

[64] Kirchhamer, C and Davidson, E (1996). Spatial and temporal information processing in the sea urchin embryo: Modular and intermodular organization of the CYIIIA gene cis-regulatory system. Development, 122: 333-348

[65] Yuh, CH and Davidson, EH (1996). Modular cis-regulatory organization of Endo16, a gut-specific gene of the sea urchin embryo. Development, 122: 1069-1082

[66] Roush, W (1996). Divide and confer: How worm embryo cells specialize. Science, 272: 1871

[67] Sagerstrom, CC, Grinblat, Y and Sive, H (1996). Anterioposterior patterning in the zebrafish, *Danio rerio*—an explant assay reveals inductive and suppressive cell interactions. Development, 122: 1873-1883

[68] Carr, AM (1996). Checkpoints take the next step. Science, 271: 314-315

[69] Carr, AM and Hoekstra, MF (1995). The cellular responses to DNA damage. Trends in Cell Biology, 5: 32-40

[70] Giulio, RT, Washburn, PC, Wenning, RJ, Winston, GW and Jewell, CS (1989). Biochemical responses in aquatic animals: A review of determinants of oxidative stress. Environmental Toxicology and Chemistry, 8: 1103-1123

[71] Shugart, L (1988). Quantitation of chemically induced damage to DNA of aquatic organisms by alkaline unwinding assay. Aquatic Toxicology, 13: 43-52

[72] Shugart, L, Gustin, M, Laird, D and Dean, D (1989). Susceptibility of DNA in aquatic animals to strand breakage: Effect of x-ray and gamma radiation. Marine Environmental Research, 28: 339-343